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Mallard Reproductive Testing in a Pond Environment: A Preliminary Study

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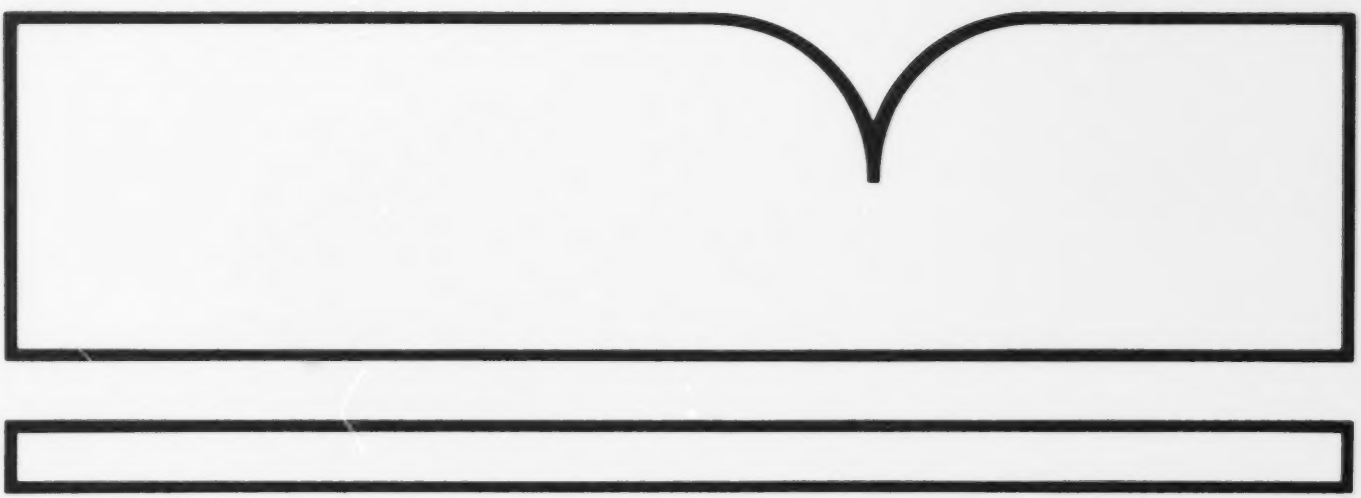

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16. ABSTRACT

A 2-year preliminary study was conducted on mallard ducks to determine the feasibility of using outdoor pond enclosures for reproductive studies and to evaluate the effects of the insecticide chlorpyrifos on mallard reproduction. No significant reproductive effects were observed for mallards receiving 8 ppm (mg/kg of feed) chlorpyrifos in their diet. Birds receiving 80 ppm chlopyrifos hatched significantly ($p<0.05$) fewer ducklings per successful nest (5.8) than controls (10.2). None of the ducklings on treatment ponds survived to 7 days. Control birds produced 8.4 ducklings per successful nest surviving to 7 days or longer. Birds in the 80 ppm treatment group consumed less feed than did controls ($p< 0.01$). Weight loss from reduced feed consumption did not occur to the extent expected, indicating that birds supplemented their diets with natural foods found in and around the ponds. In spite of relatively low treated feed consumption, brain acetylcholinesterase was significantly ($p < 0.05$) depressed (57% of controls) for 80 ppm treated birds. Studies on indoor penned mallards fed 80 ppm chlorpyrifos in their diet also resulted in acetylcholinesterase depression to the same extent, but at much higher feed consumption levels. The study demonstrated the potential of using outdoor pond enclosures to evaluate chemical effects on food consumption, brain acetylcholinesterase, and reproduction in mallards. Improvements to the study design and to the pond enclosures are discussed.

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Mallard Reproductive Testing in a Pond Environment: A Preliminary Study

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Abstract. A 2-year preliminary study was conducted on mallard ducks (*Anas platyrhynchos*), to determine the feasibility of using outdoor pond enclosures for reproductive studies and to evaluate the effects of the insecticide chlorpyrifos on mallard reproduction. No significant reproductive effects were observed for mallards receiving 8 ppm (mg/kg of feed) chlorpyrifos in their diet. Birds receiving 80 ppm chlorpyrifos hatched significantly ($p < 0.05$) fewer ducklings per successful nest (5.8) than controls (10.2). None of the ducklings on treatment ponds survived to 7 days. Control birds produced 8.4 ducklings per successful nest surviving to 7 days or longer. Birds in the 80 ppm treatment group consumed less feed than did controls ($p < 0.01$). Weight loss from reduced feed consumption did not occur to the extent expected, indicating that birds supplemented their diets with natural foods found in and around the ponds. In spite of relatively low treated feed consumption, brain acetylcholinesterase was significantly ($p < 0.05$) depressed (57% of controls) for 80 ppm treated birds. Studies on indoor penned mallards fed 80 ppm chlorpyrifos in their diet also resulted in acetylcholinesterase depression to the same extent, but at much higher feed consumption levels. The study demonstrated the potential of using outdoor pond enclosures to evaluate chemical effects on food consumption, brain acetylcholinesterase, and reproduction in mallards. Improvements to the study design and to the pond enclosures are discussed.

The U.S. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA: Pub. L. 86 Stat. 973; 90 Stat. 751; 7 U.S.C. 136 *et seq.*) requires standard lethality studies (*i.e.*, median lethal dose, LD_{50}, or median lethal concentration, LC_{50}) be performed as part of the registration process for new chemicals. In addition, the Toxic Substance Control Act (TSCA: Pub. L. 94-469, 90 Stat. 2003 15 U.S.C. 2601 *et seq.*) and FIFRA have provisions which permit the U.S. Environmental Protection Agency (EPA) to require in-depth studies into other potential environmental hazards. One such recommended study is a reproductive test conducted with bobwhite quail (*Colinus virginianus*) and mallard ducks (*Anas platyrhynchos*). Typically, these studies are conducted in indoor laboratory situations. This is desirable for both environmental control and convenience, but there is little evidence that these studies provide information comparable to exposure situations in the wild. Enclosed ponds have been used successfully to document reproductive data on mallards that were not otherwise obtainable from completely open field studies (Swanson *et al.* 1986). A 2-year preliminary study was initiated to determine the feasibility of using outdoor pond enclosures for reproductive testing on mallards.

Chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate (CAS 2921-88-2)] was selected as the test chemical; an organophosphate insecticide used on wheat, turf, mint, cotton, water (mosquito larvae), and home gardens to control a wide variety of insect pests (Kenaga *et al.* 1974; Rinehold and Witt 1984). The wide and varied use of chlorpyrifos presents ample opportunity for waterfowl to contact this chemical; it was also selected, because little information existed on the reproductive effects of this chemical on mallards, and testing has been conducted at our facility on indoor penned mallards (Gile and Meyers 1986).

Methods

The ponds were approximately 0.1 ha (maximum depth of each pond ranged between 2.4–4.9 m) surrounded by a band of emergent vegetation, primarily cattails (*Typha latifolia*), and rushes (*Juncus* sp.). Each pond was enclosed by a 1.2 m high mesh wire fence with a 0.6 m corrugated metal base. The fence was approximately 3.6 m from the edge of the water at the nearest point providing a continous band of vegetation around each pond for nesting and loafing. Water flow for each pond was maintained from 19 to 38 L/min. With the exception of the fence to exclude predators and contain study birds, the ponds were maintained as natural as possible and practical.

In 1984, groups of 4 hens and 4 drakes were randomly placed within each of 3 pond enclosures. Each bird was weighed, leg banded, and wing-clipped prior to release. After a 2-week acclimation period, birds on two randomly selected treatment ponds received game bird layer mix (Purina)[1] with either 8 or 80 ppm (mg/kg of feed) technical grade chlorpyrifos with 2% corn oil *ad libitum*, beginning 2 April 1984 through 29 June 1984. Eighty ppm is approximately 10% of the LC_{50} for 14-day-old mallards (Gile *et al.* 1983). The remaining pond was designated as the control. Birds on the control pond were provided the same feed mixtures treated with 2% corn oil only. Feed was presented to the ducks in metal canister-type poultry feeders housed in a 0.9 m × 0.9 m × 0.6 m feed shelter (4 shelters per pond). Each bird had access to all feeders within a pond. Feed consumption and spillage were monitored weekly. In addition, the pond environments provide a variety of aquatic plants and invertebrates as alternative foods.

Hens were given a free choice between using nest boxes 40 cm × 30 cm × 30 cm and nesting on the ground. Opportunity was given to each hen to nest, incubate, and hatch out broods with minimal disturbance from investigators. Number of eggs laid, number of eggs hatched, and duckling survival were recorded. In addition, data on number of nest initiations, abandonments, and depredations were recorded.

In 1985, birds were treated the same as the previous year with minor modifications. The study was started earlier in the year to permit a longer prelaying exposure period. Birds were randomly assigned to each of 5 ponds in groups of 2 males and 4 females. Three of the ponds were randomly selected as treatment ponds and 2 as controls. Birds on treatment ponds received maintenance game bird feed (Purina) with 80 ppm technical grade chlorpyrifos with 2% corn oil *ad libitum*, beginning 15 January 1985 and until hens began to lay. Once laying began, layer mix treated with chlorpyrifos was provided. Control birds received the same feed mixtures as treated birds with 2% corn oil only. Two feed shelters per pond were used in 1985. Feed consumption and spillage were monitored every 2 to 3 weeks until termination (June 1, 1986).

The study was terminated for both years when the last clutch on each pond hatched and ducklings were 7 days old. All adults were sacrificed and necropsied. Because chlorpyrifos is known to depress brain acetylcholinesterase, brain tissue samples were taken from each adult and preserved in liquid nitrogen for future acetylcholinesterase assays. These assays were performed on a Perkin-Elmer Lamba 1 UV/VIS spectrophotometer. Brain tissues were prepared for assay, using a modification of the technique described by Ellman *et al.* (1961).

[1] Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Statistical Analysis System programs (ANOVA and GLM, SAS Institute, Inc., 1982) or Student's *t* tests were used to perform analysis on feed consumption, acetylcholinesterase assays, and adult weight changes. Student's *t* tests were used to determine differences between treatment means and sexes. Student's *t* tests were used to analyze reproduction data (Sokal and Rohlf 1981). Statistical comparisons were not made between years.

Results and Discussion

Modification in study design between years did not permit statistical comparisons between years. Data from 1984 will be discussed where appropriate for comparisons between years in support of observed trends. No negative reproductive effects were found for mallards exposed to 8 ppm chlorpyrifos. In contrast, 80 ppm treated birds showed significant differences from controls in feed consumption, weight change, brain acetylcholinesterase activity, and reproduction. The results demonstrated a no-effect level between 8 and 80 ppm compared to Kenaga (1974), who concluded that the no-effect level (avian reproduction) for chlorpyrifos was between 30 and 300 ppm.

The mean daily intake of feed for treated birds at the 80 ppm level was significantly less than controls ($p < 0.01$) for both years (Table 1). The 80 ppm treated birds consistently consumed less feed than controls at each feed sampling period. Based on data from Piccirillo and Quesenberry (1979), adult mallards were expected to consume approximately 120 g/bird/day during the study. Although feed consumption of control and 8 ppm groups approximated these expected levels, birds given 80 ppm treated feed consumed much less than expected (significantly less than controls, $p < 0.01$). Birds receiving 80 ppm chlorpyrifos lost weight (significantly less than controls, $p = 0.01$; Table 2), but the decrease was not as severe as expected based on the decreased feed consumption. Eighty ppm treated birds tested in indoor pens (Gile and Meyers 1986) consumed more feed (120 g/bird/day) than treated birds on the ponds (approx. 72 g/bird/day); however, because of uncontrolled wastage inside, direct comparisons between indoor/outdoor food consumption are not appropriate. There was approximately a 17% weight loss for indoor treated birds but only 8% (significantly less than indoor birds, $p < 0.01$) for treated pond birds. Apparently, birds on the ponds were supplementing their diet with natural foods. This was particularly true of birds in the 80 ppm treated group, indicating some aversion to chlorpyrifos-treated feed.

In spite of relatively low feed consumption, 80 ppm treated birds (1985) consumed a sufficient

Table 1. Effects of chlorpyrifos on feed consumption by mallards during reproduction

Treatment	Year	Feed consumption		
		Number of feed samples	$\bar{x}$ (SE)[a,b] g/b/d	Chlorpyrifos Consumed (mg)[c]
Control	1984	25	107 (2)	0
8 ppm	1984	23	119 (4)	76
80 ppm	1984	25	67 (2)*	463
Control	1985	14	113 (7)	0
80 ppm	1985	21	78 (3)*	800

[a] The mean g/bird/day of feed eaten corrected for food wastage. Values in parentheses are standard errors
[b] Within year means with asterisks are significantly different ($p < 0.01$) from controls
[c] Estimated amount of chemical consumed over the entire length of the study based on mean g/b/day

Table 2. Effects of chlorpyrifos on adult mallard body weight and weight change

Treatment	Year	Sex	Body weight[a]				Weight change $\bar{x}$ (SE) (%)[c]
			Initial		Final		
			$\bar{x}$ (SE) (g)	n	$\bar{x}$ (SE) (g)[b]	n	
Control	1984	Male	1195 (32)	4	1419 (18)	4	+19 (4)
		Female	948 (48)	4	1138	1	+17
8 ppm	1984	Male	1053 (25)	4	1327 (41)	4	+26 (6)
		Female	1141 (36)	4	1116 (10)	4	−2 (8)
80 ppm	1984	Male	1171 (38)	4	1090	1	−7
		Female	1015 (51)	4	919 (38)	3	−6 (8)
Control	1985	Male	1207 (105)	4	1311 (79)	4	+10 (7)
		Female	1183 (59)	8	1275 (68)	6	+8 (5)
80 ppm	1985	Male	1280 (41)	6	1149 (31)	6	−10 (1)
		Female	1121 (51)	12	1057 (35)	7	−5 (4)

[a] Values in parentheses are standard errors
[b] In 1985, 80 ppm treated mallards had significantly lower final weights than controls ($p < 0.01$), males = females within treatment groups. Only 80 ppm males (1985) had final weights significantly lower than initial weights ($p < 0.025$)
[c] Mean percent weight change = mean of the differences between final and initial weight/initial weight. In 1985, % change in body weight was significantly different between 80 ppm treated group and controls ($p < 0.01$)

amount of chlorpyrifos to significantly reduce acetylcholinesterase levels to approximately 57% of control values (Table 3). For indoor penned mallards receiving 80 ppm chlorpyrifos in their diet, acetylcholinesterase levels were also depressed 43% (Gile and Meyers 1986). In 1985, mean brain acetylcholinesterase levels for 80 ppm treated females were significantly lower than similarly treated males. Because of the small sample size in 1984, differences in acetylcholinesterase were not tested. However, trends were similar for both years.

Nest depredation was relatively low in 1984, with only two nests destroyed. As a result of persistent nest predators in 1985, the number of nests attempted was high (29 for control, 17 for 80 ppm treated; Table 4). Of the 46 nests produced in 1985, 67% were destroyed by predators. In addition, predators killed 3 hens in 1984 and 5 hens in 1985. These losses confounded egg production data but did not obscure possible effects of chlorpyrifos on the other reproduction parameters monitored (Table 4). There were no significant differences in eggs produced per successful nest between controls and either treatment. However, the mean number of ducklings hatching per nest was significantly different ($p < 0.05$) between controls ($\bar{x} = 10.8$) and 80 ppm treated birds ($\bar{x} = 5.8$) in 1985. While there was no significant difference observed in 1984, the 80 ppm group provided only 1 nest for comparison. Indoor testing with mallards (Gile and Meyers 1986) showed that chlorpyrifos at 80 ppm in feed caused a significant reduction in total eggs produced but not in fertility or hatchability. Both 8 ppm and control birds (both years) also produced more ducklings that survived to 7 days or longer

Table 3. Effects of chlorpyrifos on brain acetylcholinesterase levels[a]

		Control			8 ppm				80 ppm			
		$\bar{x}$	(SE)[b]	n	$\bar{x}$	(SE)	% of control	n	$\bar{x}$	(SE)	% of control	n
1984												
	Male	2.68	(0.13)	3	2.63	(0.07)	98	4	1.60		60	1
	Female	2.54		1	2.49	(0.02)	98	4	1.26	(0.13)	50	3
1985[c]												
	Male	2.46	(0.10)	4					1.55	(0.10)	63	6
	Female	2.40	(0.06)	6					1.29	(0.14)	54	7

[a] As measured by mean number of μmoles acetylthiocholine iodide hydrolyzed/mm/g tissue
[b] Values in parentheses are standard errors
[c] In 1985, 80 ppm treated mallards had significantly lower ($p < 0.05$) acetylcholinesterase levels than controls. Females in the 80 ppm group had significantly lower ($p < 0.05$) levels than males in that group

Table 4. Reproductive effects of chlorpyrifos on mallards

Treatment	Hen-days[a]	Total eggs produced	Number successful nests	Mean number eggs/Successful nest	Mean number eggs hatched/ Successful nest[b]	Number mean ducklings surviving ≥7 days/ Nest
1984						
Control	336	69	4	12	6.8	6.2
8 ppm	336	63	3	11.7	9.3	8.6
80 ppm	251	15	1	7	7.0	0
1985						
Control	1174	252[c]	5	10.8	10.2	8.4
80 ppm	1163	139	5	7.8	5.8	0

[a] Combined total number of days all hens were present on ponds; differences within a year reflect loss of hens to predation
[b] Mean number of eggs hatched/successful nest was significantly different ($p < 0.05$) between control and 80 ppm treatment in 1985
[c] Total eggs produced in 1985 was affected by heavy nest predation because hens continually replaced those destroyed by predators

than did 80 ppm birds (0 birds both years). Six ducklings produced in 1985 by an 80 ppm treated hen died within the first 24 hr because the hen was killed by a predator. If all 6 had survived to 7 days there would have only been 1.2 ducklings surviving per successful nest in the 80 ppm group compared to 8.4 for controls. It should not be overlooked that ducklings on treatment ponds were also exposed to the same chlorpyrifos diet as adults. Although it is impossible to compare or even separate direct effects of the chemical on ducklings with those effects attributable to treated parents, the observations indicated that 80 ppm chlorpyrifos treated hens were not attentive to their offspring, which resulted in their death within the first 48 hours.

Studies by Hurlbert *et al.* (1970) suggested another possible explanation for duckling mortality; control ducklings, as well as those on ponds sprayed with chlorpyrifos, had difficulty finding adequate quantities of "wild foods" after feeders were removed, which resulted in considerable loss of weight. In addition, experience has shown that ducklings will avoid chlorpyrifos treated food (Gile *et al.* 1985). In the present study, avoidance of treated feed and difficulty in finding "wild foods" may have resulted in starvation or increased stress experienced by ducklings on treatment ponds. These conditions alone or in combination with those mentioned above contributed to high duckling mortality on treatment ponds.

Conclusions

Ponds appear to provide a more realistic assessment of field effects by providing free choice of food, nesting conditions, and more natural environmental stresses. Reproductive effects caused by chlorpyrifos fed to mallards on outdoor ponds were evident in this study at 80 ppm. This modified the conclusions of Kenaga (1974), who determined that the no effect level of chlorpyrifos on avian repro-

duction was between 30 and 300 ppm. The no-effect level for mallard reproduction was determined in the present study to be between 8 and 80 ppm.

The similarities observed between ponds and indoor pens in food consumption, weight change, acetylcholinesterase depression, and egg production/clutch size indicate that with some minor modifications to the study design (more replicates) and to the pond enclosures, these systems can complement indoor reproductive tests. In addition, continued research with pond environments, such as those described in the present study and elsewhere, will contribute to the development of field testing protocols for new chemicals.

Predation was a serious problem throughout this study. The success of further studies using pond enclosures will require better control over predators. Mammalian predators were the most serious problem. It has since been found that an electric fence around each pond is an inexpensive deterrent for mammalian predators. Avian predators killed several ducks during or shortly after the two week acclimation period. With time, the ducks learned to avoid avian predators. In future studies, it may be necessary to extend the acclimation period until ducks presumably could learn to avoid avian predators. Individuals lost during this time would be replaced. Flight obstructions or other methods to discourage avian predators are possible. If feasible, future studies should include more true replicates (*i.e.*, more ponds).

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